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TESTS ON SHORT-TERM STORAGE OF PACIFIC HALIBUT (*Hippoglossus stenolepis*) SPERM AND SALINITY TOLERANCE OF PACIFIC HALIBUT AND SABLEFISH (*Anoplopoma fimbria*) SPERM

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ABSTRACT

Bowden, D. G., E. P. Groot, and J. O. T. Jensen. 1990. Tests on short-term storage of Pacific Halibut (Hippoglossus stenolepis) sperm and salinity tolerance of Pacific Halibut and Sablefish (Anoplopoma fimbria) sperm. Can. Tech. Rep. Fish. Aquat. Sci. 1725: 17 p.

The effect of short-term storage on the duration of forward motility (FM) of sperm from Pacific halibut (Hippoglossus stenolepis) as well as the salinity tolerance of halibut and sablefish (Anoplopoma fimbria) sperm were examined. Milt was collected from spermiating halibut and sablefish using routine techniques. Halibut milt was stored in sealed plastic bags at 3.3±0.35°C (± SD) and examined daily for duration of FM. Salinity tolerance was determined by mixing fresh milt in various sea water concentrations (5-55‰ for halibut sperm and 10-60‰ for sablefish sperm) and then examining the duration of FM of spermatozoa. Halibut sperm was successfully stored for up to 195.0 h. Halibut sperm exhibited FM when activated with sea water ranging from 10-50‰ and sablefish sperm demonstrated FM in salinities ranging from 15-60‰.

RESUME

Bowden, D. G., E. P. Groot, and J. O. T. Jensen. 1990. Tests on short-term storage of Pacific Halibut (Hippoglossus stenolepis) sperm and salinity tolerance of Pacific Halibut and Sablefish (Anoplopoma fimbria) sperm. Can. Tech. Rep. Fish. Aquat. Sci. 1725: 17 p.

Les auteurs ont examiné les effets du stockage à court terme sur la durée de la mobilité des spermatozoïdes chez le flétan du Pacifique (Hippoglossus stenolepis) dès le début de l'entreposage de la laitance ainsi que la tolérance à la salinité de la laitance du flétan et de la morue charbonnière (Anoplopoma fimbria). Ils ont recueilli la laitance des flétans et des morues en spermiogenèse au moyen de techniques courantes. La laitance des flétans a été entreposée dans des sacs en plastique scellés à 3,3°C (avec un écart-type de 0,35°C) et a été examinée quotidiennement pendant toute la période où les spermatozoïdes sont demeurés mobiles. Pour déterminer la tolérance à la salinité, on a mélangé de la laitance fraîchement recueillie avec diverses concentrations d'eau de mer (5-55‰ pour la laitance des flétans et 10-60‰ pour la laitance des morues), puis on a observé la durée de mobilité des spermatozoïdes à partir du moment où la laitance a été stockée. On a pu entreposer la laitance des flétans sans mettre fin à la mobilité des spermatozoïdes pendant 195,0 heures. La mobilité des spermatozoïdes des flétans a été stimulée avec de l'eau de mer d'une concentration de 10 à 50‰, et la mobilité des spermatozoïdes de la morue, avec de l'eau de mer d'une concentration de 15 à 60‰.

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INTRODUCTION

There has been a great deal of interest in sablefish (Anoplopoma fimbria) and Pacific halibut (Hippoglossus stenolepis) mariculture in recent years. Investigations at the Pacific Biological Station, Nanaimo, B.C., Canada, have dealt primarily with sablefish. Preliminary observations on sablefish gamete storage, incubation and embryonic development were made by Alderdice et al. (1988a). Further studies on sablefish and halibut incubation and developmental biology are currently in progress (McFarlane et al. MS). The fertilization process of these two species is still not fully understood. Therefore, a series of tests were conducted to learn more about the effects of short-term storage and exposure to various salinities on sperm.

Spermatozoa of fishes are immotile in seminal fluid. Hence, since energy is not required for locomotion in this fluid, sperm are well suited to short-term storage (Stoss and Donaldson 1982, Stoss 1983). Stoss and Donaldson (1982) outline some of the criteria for successful short-term storage of fish sperm. The most notable of these criteria are (1) reduction in temperature of sperm and (2) provision of gaseous exchange. Optimum storage temperatures are above 0°C and usually in the range of 2-6°C (Stoss and Donaldson 1982, Stoss 1983, Jensen and Alderdice 1984, Alderdice et al. 1988a and b).

Short-term storage of sperm at temperatures below 0°C is not generally practised, since storage at subzero temperatures requires the use of special extending media and cryoprotectants. However, several authors have successfully used cryogenic techniques to preserve the sperm of various marine fishes (Pacific herring, Blaxter 1953; Atlantic cod, Mounib et al. 1968; plaice, Pullin 1972; grouper, Withler and Lim 1982; and Atlantic halibut, Bolla et al. 1987). Of particular interest is the work by Bolla et al. (1987) on the long-term storage of Atlantic halibut (Hippoglossus) sperm. Halibut sperm were stored for 14 days in liquid nitrogen (approx. -196°C). Thawed sperm showed good motility and fertilized fresh eggs equally as well as the control (fresh) sperm.

Several investigators have examined the use of extending media during short-term storage and gamete dilution (Billard 1978, Stoss 1983). Billard (1978) suggested that sea water was not the best medium for sperm dilution. He observed better results using the physiological extender DCSB4 (NaCl, 19.5 g/l; glycine, 6.25 g/l; $MgSO_4 7H_2O$, 0.26 g/l and $CaCl_2 2H_2O$, 0.25 g/l). This extender increased the duration of motility of 2 species of marine fish (sea bass and sea bream) sperm by a factor of 3 times over that induced by sea water. The use of artificial and natural extending media in artificial insemination techniques is discussed by Stoss (1983).

Many investigators (Hwang and Idler 1969, Hines and Yashouv 1970, Billard 1978, Morisawa and Suzuki 1980) have examined the roles of various ions and osmolality in the initiation of sperm motility. Stoss (1983) and Morisawa (1987) provide detailed accounts of the processes of sperm activation. The sperm of marine fishes are activated by an increase in osmolality of the medium external to the sperm cells (Morisawa 1987).

The objectives of this study were to (1) develop an experimental procedure suitable for the examination of fish sperm motility, (2) obtain preliminary data on the short-term storage of Pacific halibut sperm, and (3) determine the salinity tolerance for halibut sperm and sablefish sperm.

MATERIALS AND METHODS

BROOD STOCK

Halibut were caught by longline, during 1987 and 1988, in Queen Charlotte Sound, B.C. Sablefish were captured by Korean-style conical traps, during September 1988, near Ucluelet on the west coast of Vancouver Island, B.C. The fish were transported to the Pacific Biological Station by boat (halibut) and aerated tanker truck (sablefish). Prior to the experiments the halibut were held in large fibreglass tanks and the sablefish in large wooden tanks. Fish were maintained on diets of frozen herring and squid. Sex was determined by inserting a cannula into the genital pore and examining the extracted, immature gametes. Male and female fish were kept separate to prevent premature induction of spawning.

GAMETE COLLECTION AND STORAGE

Halibut and sablefish (Table 1) were spermiating several weeks prior to the start of the experiment. The fish were anaesthetized with tricaine methane sulfonate (MS-222, 75-100 mg/l sea water) in preparation for gamete collection. Excess water was wiped from the abdomen and genital pore, before collecting milt. Halibut milt (20-30 ml) was collected into plastic bags (Whirl-pac; Pioneer Container Corp., Cedarburg,

Wis., USA) by applying gentle pressure along the abdomen. These bags were sealed to include a large volume of air and then stored on their side, to allow maximum milt to air ratios (Richter and Goos 1982, Stoss 1983, Jensen and Alderdice 1984). Milt was kept cool by placing the bags in a metal tray floated on a water bath (mean temp. = 3.3 $\pm$ 0.35°C). The sperm motility procedure (described above) was conducted for sperm from two male halibut. The subsampling procedure was carried out daily until sperm no longer exhibited FM when mixed with sea water. Three replicate measurements were obtained per fish for each day of sperm storage. Sablefish milt was collected in a similar manner, though it was only stored for one hour prior to testing.

PREPARATION OF TEST SALINITIES

A series of salinities (nominal concentrations of 5, 10, 15, 20, 30, 35, 40, 50, 55, 60 and 65‰) were prepared from a stock solution either by serially diluting with distilled water, or by adding sea salt (Forty Fathoms; Marine Enterprises, Towson, Md, USA). The initial stock solution was prepared by adding sea salt to the filtered, laboratory sea water supply (29.5‰). The salinities of these test solutions were determined with a digital chloridometer (Haake Buchler Instruments Inc., Saddle Brook, NJ, USA).

GAMETE MOTILITY TESTING PROCEDURES

Preliminary observations on the duration of forward motility (FM) of halibut sperm revealed that halibut sperm remained motile for a relatively long period (> 5 min initially). Due to the length of duration of FM of halibut sperm it was felt that observations made on a single sample of sperm placed on a microscope slide with a cover slip would not yield highly variable results. Terner and Korsch (1963) show that the duration of motility of sperm under a cover slip is artificially limited. These investigators examined trout sperm and found that diluted sperm stored in an open vessel retained motility for 1/2 h after mixing. However, sperm became immotile after 30 s when examined under a cover slip. Therefore, the following technique was devised.

All experimental procedures were conducted in a refrigerated room (5°C air temp.) and all test apparatus (microscope, pipettes, beakers and slides) and materials used

(test salinities) were precooled prior to testing. Milt was mixed thoroughly to provide homogeneous samples. Sperm were activated by adding 1 part milt with 100 parts sea water (Alderdice et al. 1988b). Milt (10 µl) was drawn into a positive displacement pipette and then expelled into 1.0 ml sea water (34.4‰ salinity) in a 2.0 ml beaker. The milt and sea water were mixed for 10 s to produce a uniform cell suspension. Subsamples (20 µl) of the sperm suspension were taken from the beaker, placed on microscope slides and covered with cover slips. These subsamples were examined under high power (1260x) using light microscopy. Fresh subsamples were taken at one minute intervals until FM ceased. The sperm - sea water solution was stirred each time prior to subsampling. The duration of sperm motility was assessed as the time from mixing of milt and sea water until an estimated 95% of the sperm cells no longer exhibited FM. In addition, qualitative assessment of the vigor of sperm was made.

HALIBUT SALINITY TOLERANCE

Observations were made on the salinity tolerance of halibut sperm. Sperm were mixed with the sea water solutions of the various salinities described above (i.e., 5 - 65‰) and examined for FM to determine the upper and lower limits of salinity tolerance. The sampling and FM examination procedure was similar to that used in the halibut gamete storage experiment.

SABLEFISH SALINITY TOLERANCE

Fresh sablefish milt, stored for up to 1 h., was mixed with the prepared salinity treatments. The sampling and Fm determination technique was the same as that used for halibut milt except for the following modification. Preliminary observations showed a shorter duration (approx. 2 min.) of sperm motility in sablefish than in halibut. Therefore, a single subsample of the sperm - sea water suspension was examined for the duration of sperm motility rather than repeated sampling from the same suspension. Three replicate measurements were made for each fish at each test salinity. Two male sablefish were tested. A qualitative assessment of sperm vigor also was made.

RESULTS

HALIBUT GAMETE STORAGE

Experiments on short-term gamete storage showed that halibut sperm stored at 3.3 ± 0.35°C (mean temperature ± SD) retained the ability to be activated for up to 195.0 h of elapsed storage time (EST) (Table 2). Although variability in the duration of FM for halibut sperm was high, there was a trend of gradual reduction in duration of motility over time (Fig. 1). By 215.7 h EST, FM was not observed in sperm samples (Table 2). There was a great deal of variability in the duration of FM between replicates and between storage times (Fig. 1). Initially all activated sperm cells demonstrated high vigor of motility at each sampling time (0-195.0 h). However, sperm vigor within a test sample decreased from high to moderate following 2 min. of observation, after which vigor decreased slowly. In addition, initial observations of fresh milt from both males tested showed approximately 50% immotile cells prior to storage.

HALIBUT SALINITY TOLERANCE

Observations of salinity tolerance for halibut sperm, although only preliminary, showed that sperm were activated and exhibited FM in sea water ranging from about 10-50‰ salinity. Whereas, no activation or FM occurred at salinities of 5 and 55‰. Observations regarding the duration or vigor of motility were not made for these tests.

SABLEFISH SALINITY TOLERANCE

The more extensive data on the salinity tolerance of sablefish sperm indicated that sperm were activated by sea water ranging from 15‰ to 60‰ salinity but exhibited no signs of activation or FM in salinities of 10 and 65‰ (Table 3). The duration of FM was highly variable and did not vary significantly ($p < .05$) between 15‰ and 60‰ (Fig. 2). Initial vigor of FM appeared similar at all test salinities and decreased quickly from high to low (< 1 min.) within an individual test. There did not appear to be a significant difference in duration of FM between the two fish examined.

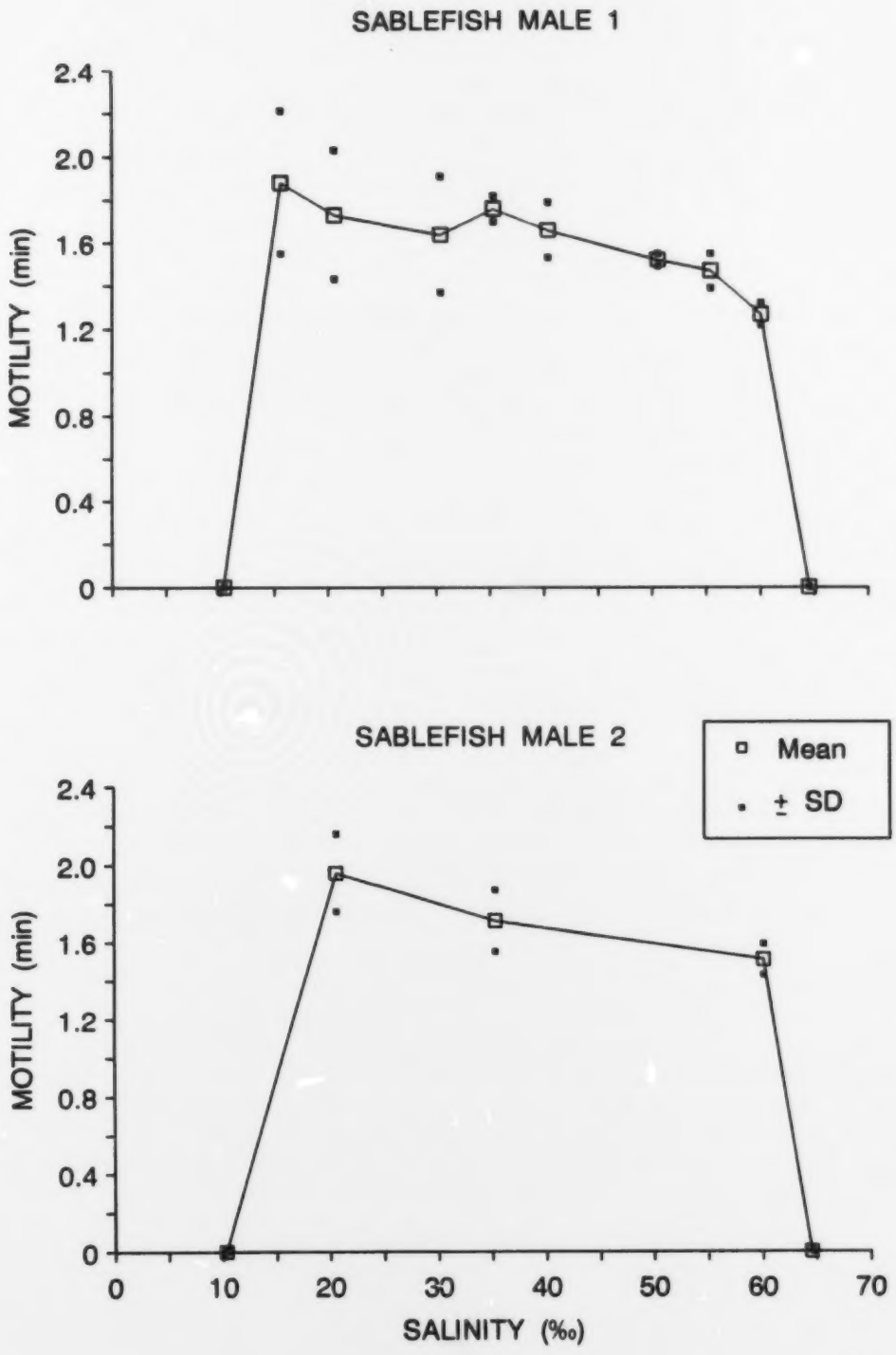

Fig. 1. Duration of forward motility (mean FM ± SD, min.) of Pacific halibut sperm after various periods of storage (h.).

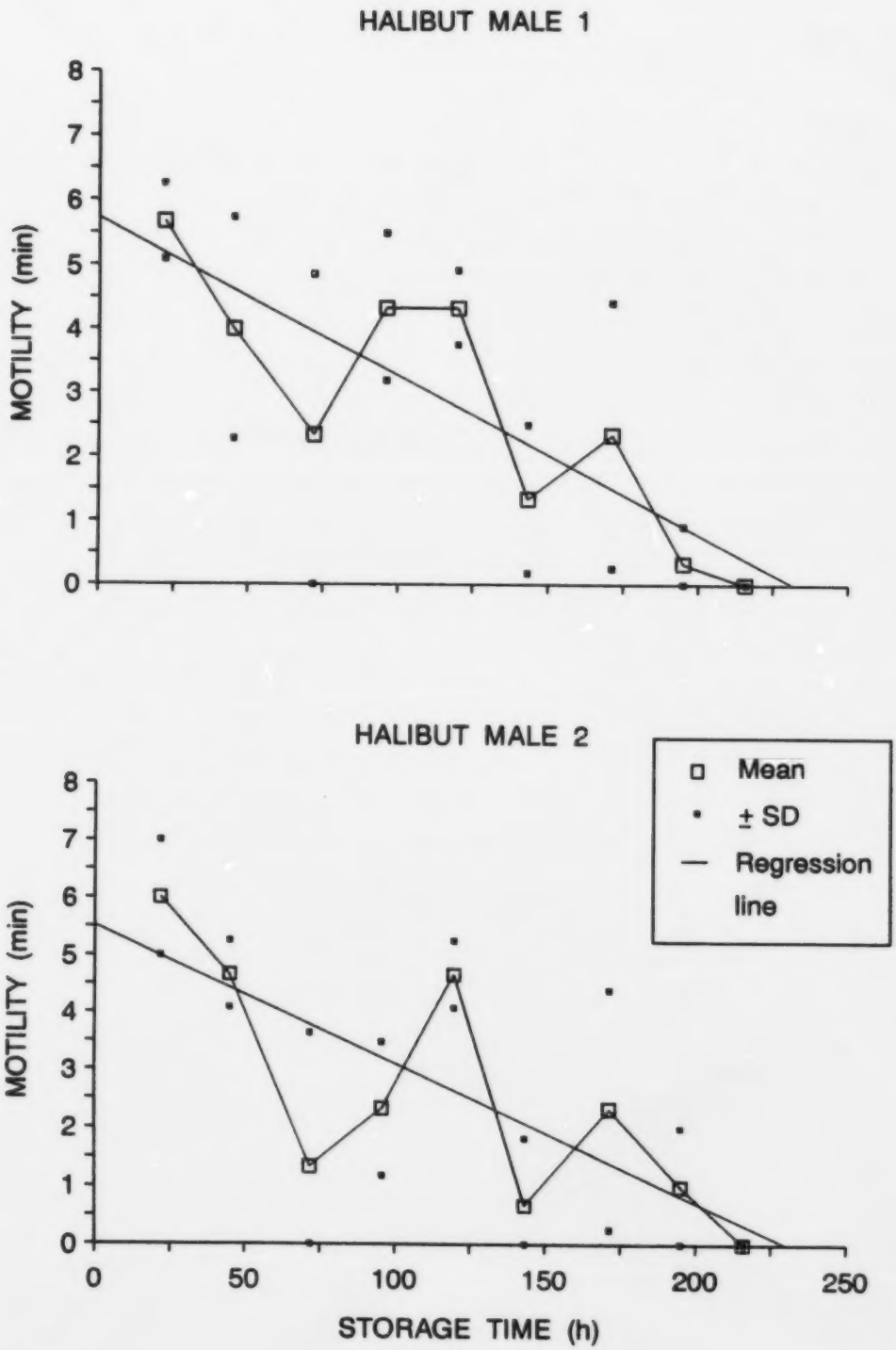

Fig. 2. Duration of forward motility (mean FM ± SD, min.) of sablefish sperm activated in various salinities (‰).

DISCUSSION AND CONCLUSIONS

The gamete storage tests showed that halibut sperm can be stored at 3.3°C, for at least 8 days and retain the capacity for FM when activated. In a similar experiment, Alderdice et al. (1988a) found that sablefish sperm demonstrated high motility after 13 days of storage at 3°C. A second series of experiments by the same researchers showed high motility in spermatozoa after 20 days of storage at 2-6°C. Hence, it appears that halibut sperm does not store as well as sablefish sperm. However, more tests with more samples of milt and various storage temperatures will be necessary to verify this conclusion.

The salinity tolerance results from this study showed that halibut and sablefish sperm have similar tolerance to a wide range of salinities. The two species varied only slightly in sperm motility response over the range of salinities that activate sperm. Halibut sperm were activated over the range of 10-50‰ and sablefish sperm were activated at 15-60‰. Morisawa and Suzuki (1980) found that puffer fish (Fugu niphobles) sperm were motile in sea water above 300 mosm/kg (approx. 10-39‰) and that maximum sperm motility occurred at 400 mosm/kg (approx. 13‰). Billard (1978) examined the effect of sperm dilution on motility using media of various salinities. He showed that the sperm from sea bass (Dicentrarchus labrax) and sea bream (Sparus auratus) were motile only in salinities above 10‰ and that the optimal salinity for dilution of sea bass sperm was 20‰. Hines and Yashouv (1971) observed a range of salinity tolerance for the grey mullet (Mugil capito) similar to our findings, with sperm motility observed up to 48‰. However, their results show a gradual increase in duration of motility with increasing salinity levels from about 8 to 35‰.

In the tests described herein, both halibut and sablefish sperm show a wide range of salinity tolerance. The results from the sablefish tests (Fig. 2) suggest that an optimum salinity for duration of motility may occur near 20‰, similar to that reported for sea bass and sea bream (Billard 1978). Halibut and sablefish are bathypelagic and normally spawn at depths of greater than 200 m. Therefore, the salinity that they would normally be exposed to is about 35‰. Hence there appears to be no physical advantage for increased duration of sperm motility at a lower salinity.

The question arises as to the correlation between FM and viability or fertilization success. Fertilization rates of sablefish sperm and eggs stored at 3°C were examined by Alderdice et al. (1988b). They observed relatively good fertilization

success (48.4%) after 3 days of storage, but no fertilization after day 6 of storage even though stored sperm still exhibited motility. Therefore, sperm motility does not necessarily ensure that sperm are viable and capable of successful fertilization. Several investigators (Scott and Baynes 1980) found that stored sperm, although motile, were unable to fertilize eggs. The next step in our investigation will be to conduct fertilization tests over a range of salinities. It is anticipated that the range of tolerance for fertilization success will be much narrower than the wide range of salinity tolerance that we have observed for both halibut and sablefish sperm.

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Table 1. Fish catch data for halibut and sablefish. The identification tag information is included (in brackets) for possible reference to other related studies.

Male	Date Caught	Capture Location	Length (cm)	Weight (g)
Halibut				
male 1 (yellow tag)	1987	Queen Charlotte Sound	102	-
male 2 (orange/yellow tag)	1988	Queen Charlotte Sound	103	-
Sablefish				
male 1 (red 804/white 1611)	Sept., 1989	Ucluelet	72.5	4357
male 2 (red 811/white 1615)	Sept., 1989	Ucluelet	66.6	3691

Table 2. Duration of forward motility (min.) of sperm activated in sea water of 34.4‰ salinity after various periods of storage (h.) at 3.3 ± 0.35°C (mean ± SD, n=9).

Fish No.	Storage Time (h.)	Duration of Motility (min.)				
		Rep1	Rep2	Rep3	mean	SD
Male 1	0	-	-	-	-	-
	21.8	5	6	6	5.67	0.58
	45.0	5	5	2	4.00	1.73
	71.8	2	5	0	2.33	2.52
	95.9	5	3	5	4.33	1.15
	119.8	5	4	4	4.33	0.58
	143.2	0	2	2	1.33	1.15
	171.3	0	4	3	2.33	2.08
	195.0	0	0	1	0.33	0.58
	215.7	0	0	0	0	0
Male 2	0	-	-	-	-	-
	21.8	5	6	7	6.00	1.00
	45.0	5	4	5	4.67	0.58
	71.8	0	4	0	1.33	2.31
	95.9	3	1	3	2.33	1.15
	119.8	5	4	5	4.67	0.58
	143.2	0	2	0	0.67	1.15
	171.3	0	4	3	2.33	2.08
	195.0	0	1	2	1.00	1.00
	215.7	0	0	0	0	0

Table 3. The duration of forward motility (min.) of sablefish sperm, activated in sea water of various salinities (‰).

Fish No.	Salinity (nominal) (‰)	Salinity (measured) (‰ ± SD)	Duration of Motility Rep1 (min.)	Rep2 (min.)	Rep3 (min.)	mean (min.)	SD (min.)
Male 1	10	10.31 ± 0.22	0	0	0	0	0
	15	15.66 ± 0.23	1.76	1.62	2.25	1.88	0.33
	20	20.62 ± 0.12	2.08	1.53	1.58	1.73	0.30
	30	30.46 ± 0.14	1.95	1.50	1.47	1.64	0.27
	35	35.34 ± 0.15	1.72	1.82	1.73	1.76	0.06
	40	40.44 ± 0.38	1.78	1.67	1.52	1.66	0.13
	50	50.63 ± 0.26	1.52	1.50	1.55	1.52	0.03
	55	55.53 ± 0.29	1.53	1.52	1.38	1.47	0.08
	60	60.18 ± 0.43	1.32	1.25	1.23	1.27	0.05
	65	64.51 ± 0.43	0	0	0	0	0
Male 2	10	10.31 ± 0.22	0	0	0	0	0
	20	20.62 ± 0.12	1.92	1.78	2.18	1.96	0.02
	35	35.34 ± 0.15	1.60	1.65	1.90	1.71	0.16
	60	60.18 ± 0.43	1.58	1.52	1.42	1.51	0.08
	65	64.51 ± 0.43	0	0	0	0	0